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## **Studies on the Mechanisms of Microbial Adaptation to the Physical Environment**

M. R. Heinrich, Ames Research Centre, California, USA

### **INTRODUCTION**

The environmental factors which affect humans and other animals also influence the microorganisms which are such an important part of our ecology. Some of the microorganisms are very closely associated with animals, living in the digestive tract and synthesizing essential nutrients for the host. For these microbes, most external physical changes are of little consequence, because they are well shielded by the animals' homeostatic systems. The vast majority of microorganisms, however, live free in nature, especially in the soil and oceans. It has been estimated that the upper 15 cm of a fertile soil may contain over 4000 kg of bacteria and fungi per hectare (Stanier *et al.*, 1963). These organisms are responsible for degrading the complex molecules of plants and animals when they die, eventually producing simple organics, carbon dioxide, and inorganics, which are then used for the next cycle of plant growth. It is believed that over 90 % of the biologically produced carbon dioxide results from the metabolic activity of bacteria and fungi. In addition to recycling plant nutrients, soil bacteria also provide new nutrients through 'fixation' of atmospheric nitrogen into ammonia and nitrate, the forms which can be used by plants. Microorganisms also have an enormous capacity for detoxifying both natural and man-made poisons.

All of these functions of microorganisms are essential to the operation of the material cycles on Earth. This is true of all locations on the planet, regardless of the climate or other environmental factors. In fact, one of the most impressive attributes of microorganisms is their ability to adapt to every stable environment on Earth. These include such extremes as polar regions, hot springs, water saturated with salt, mountain tops, ocean depths, acid and alkaline waters, deserts, intense radioactivity, soil and water contaminated with toxic chemicals or petroleum, and areas devoid of oxygen. Microorganisms are also found

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suspended in the atmosphere, although it has not been possible to prove that they multiply there.

The conditions found in these environments are more severe than those we usually assume can be tolerated even by cell constituents — the enzymes, nucleic acids, membranes, etc. The fact that organisms populate all those areas indicates that the structure of cell components can be modified to maintain function under severe environmental stress. These interesting organisms are ideal tools for studying those adaptations, and they have been used extensively in that way. Eventually it will be possible to correlate changes in molecular structure, cell structure, and metabolic pathways with the ability to adapt to particular physical parameters. This review will present some of the more recent work in those directions, including principally the effects of high temperature, low temperature, and pressure. Two recent books are useful references (Heinrich, 1976; Kushner, 1978).

### ADAPTATION TO HIGH TEMPERATURE

Probably the most impressive adaptation is that of bacteria to high temperature, including strains living in hot springs, hot acid waters, home and industrial hot water heaters, and decomposing organic material (Tansey and Brock, 1978). Although spores are well known to have great heat resistance, most of these *thermophilic organisms* are not spore-formers, and the resistance is inherent to the vegetative cells. Bacteria are known to inhabit boiling springs (95 °C at that altitude), and their growth rate has been measured *in situ* (Bott and Brock, 1969). Other microorganisms are not as tolerant to heat as are bacteria; photosynthetic algae (*blue-green*) are found up to *c.* 70 °C, other algae and fungi up to 60 °C, and protozoa up to 56 °C (Tansey *et al.*, 1978). It should also be pointed out that, for all these organisms, there are many different strains, with different optimum and maximum growth temperatures.

It is obvious that thermophilic organisms must differ considerably from their more common mesophilic relatives. Among the required changes for life at high temperatures would be a stabilization of enzymes and nucleic acids, and an increase in the melting point of lipids. One possibility which has been considered is that thermophilic organisms contain stabilizing factors which prevent denaturation of their enzymes and nucleic acids. Although considerable effort has been spent in looking for enzyme stabilizers, it has not been possible to prove their existence. In fact, enzymes isolated from thermophiles, purified and repeatedly recrystallized, retain their heat resistance. Thus the stability of thermophilic enzymes appears to be an inherent property of the enzyme molecule. This provides an excellent opportunity to study the factors which contribute to protein stability, by comparing the structure of

the same enzyme isolated from organisms which live at different temperatures. Prior work has been reviewed by Ljungdahl and Sherod (1976) and by Amelunxen and Murdock (1978). The stability of protein molecules may be influenced by changing the amino acid composition, or by changing the amino acid sequence. Changes of either kind will result in a protein with different charged groups, hydrophobic groups, hydrogen bonding, salt linkages, or disulfide bonds, and these will result in altered secondary structure (helicity,  $\beta$ -structure). Singleton (1976) made an extensive analysis of data for 12 enzymes isolated from 15 thermophilic and 56 non-thermophilic organisms. Although there were slight differences in the amino acid composition of the same enzyme of these two classes, there were no significant, consistent differences which were characteristic of thermophilic enzymes. Also, several calculated structural parameters (helix content, hydrophobicity index, melting point) did not show significant differences. The only property which showed a correlation was a decrease in the  $\beta$ -sheet content of most of the thermophilic enzymes. It is apparent that the heat stability of these proteins will not be explained by any of the obvious possibilities, but that detailed knowledge of sequence and 3-dimensional structure will be required.

One series of studies in that direction concerns *ferredoxin*, isolated from thermophilic and other *Clostridia* (Devanathan *et al.*, 1969). When these molecules were sequenced (Tanaka *et al.*, 1973), several differences were found; the net result of the amino acid substitutions shows thermophilic ferredoxins to have more charged groups and to be more acid. Adman, Sieker and Jensen (1973) determined the 3-dimensional structure of ferredoxin from *Micrococcus aerogenes* by X-ray diffraction; Tanaka *et al.* (1973) and Perutz and Raidt (1975) made a detailed analysis of *Clostridial* ferredoxins based on that model. They concluded, among other things, that the thermophilic ferredoxins were stabilized by salt bridges between residues near the ends of the molecule.

The ferredoxins are not ideal models for enzymes because they are only a fraction of the size, and have only limited secondary structure. Recently Harris' laboratory has reported the first complete sequence and structure (by X-ray diffraction) of a thermophilic enzyme, the glyceraldehyde 3-phosphate dehydrogenase from *Bacillus stearothermophilus* (Biesecker *et al.*, 1977). A detailed comparison with the mesophilic enzyme (from lobster muscle) shows a great deal of similarity, and some differences. An unexpected finding was that the core of the thermophilic enzyme was no more hydrophobic than that of the mesophilic enzyme. It appears that one of the principal factors in the stability of the thermophilic enzyme is its higher content of arginines, two of which form additional salt bridges between some of the four sub-units (of 333 amino acids each) which make up the active enzyme. Further

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study of this and other enzymes, as they become available, will provide more details about the factors contributing to stability. It is apparent now, however, that stability to heat is due to a summation of many subtle changes in the structure of the protein.

*Nucleic acids* are another class of macromolecules which are 'denatured' at high temperatures. In this case, heat destroys the hydrogen bonds which maintain the double helix of DNA and the complex RNA structures. These changes can be followed by measuring the increase in absorbance at 260 nm of a nucleic acid solution as it is heated; the mid-point of this curve is the transition or melting-out temperature,  $T_m$ . Not surprisingly, the nucleic acids of thermophilic organisms have  $T_m$ 's as much as 20 °C higher than those of mesophiles (for reviews see Stenesh, 1976; Reid, 1976). With DNA, the stabilization can be accounted for largely by an increase in the guanine-plus-cytosine content, with their strong hydrogen bonding, and a corresponding decrease in adenine-plus-thymine. Transfer RNAs, with their modified (or 'minor') bases, offer further opportunities for stabilization, as found by Watanabe *et al.* (1974, 1976a) in *Thermus thermophilus*, which grows at 85 °C. This organism has methionine-transfer RNA with an extra guanine-cytosine pair, as compared with the very similar tRNA from *Escherichia coli*. Even more important, however, is the stabilization due to bonding with a new base (5-methyl-2-thiouridine), probably formed by addition of sulfur to the one ribothymidine in the RNA. In fact, the amount of thio-ribothymidine in the transfer RNA was proportional to the growth temperature, suggesting that a thiolating enzyme became more active as the temperature increased (Watanabe *et al.*, 1976b).

Although no external factors have been implicated in stabilizing thermophilic proteins, this may not be true for the nucleic acids. Oshima (1975), in studying the role of polyamines in high-temperature protein synthesis, isolated the amines from *T. thermophilus*. He found spermine and a new tetra-amine,  $H(NHCH_2CH_2CH_2)_3NH_2$ , which he called thermine. DeRosa *et al.* (1976) found spermidine, thermine, and a new triamine,  $H(NHCH_2CH_2CH_2)_2NH_2$ , which they named caldine, in *Caldariella acidophila*. These polyamines may well function to stabilize nucleic acids *in vivo*, as they are known to do *in vitro*.

Lipids of animals, plants, and microorganisms have long been known to vary in composition with the environmental temperature. This is another essential adaptation for thermophilic microbes, which would otherwise contain just a droplet of melted fat. Probably the most important function of the lipids is to maintain the integrity of the cell membranes, which are vital for structure, transport, and enzyme activity. The current view is that the organism must maintain the proper lipids so that the temperature of transition between the gel and liquid-crystalline states in the membrane lipids falls near the growth

temperature (McElhaney, 1976; Esser and Souza, 1976). This is accomplished by varying the chain length, branching, and unsaturation in the fatty acids of the lipids. A more extreme change is described by DeRosa *et al.* (1977) and by Langworthy and Mayberry (1976) for thermophilic, acidophilic bacteria of the *Caldariella* group. Instead of the normal ester lipids, these organisms contain ether lipids based on a 72-member macrocyclic tetraether containing two C<sub>40</sub> phytane diols plus glycerol and nonitol. The other major components of membranes, the proteins, are discussed by Welker (1976).

### ADAPTATION TO LOW TEMPERATURE

The cold areas of the Earth include not only the polar regions and mountains, but also the greatest portion of the oceans. Living organisms are found in all these locations (Baross and Morita, 1978). As ZoBell (1962) has pointed out, more than 90 % of ocean water is colder than 5°C, and only a relatively thin layer at the surface varies in temperature with the climate. Nevertheless, microorganisms are found at all depths. Most of the investigation of the polar regions has been concentrated in the Antarctic, where every area studied has been found to contain organisms (Cameron *et al.* 1976; Uydess and Vishniac, 1976).

The organisms growing at low temperatures (*psychrophiles*) usually have their entire growth range shifted downward. Growth rates at lower temperatures (e.g. in the area of 0–15°C, depending on the organism) are generally similar to those of mesophilic organisms at 25–30°C. With both minimum and maximum growth temperatures changed, there is considerable study of just what factors set these limits (Inniss and Ingraham, 1978). It has been shown that protein synthesis, both *in vivo* and *in vitro*, is reduced when the temperature approaches the maximum. The same system appears to be limiting when tested at the minimum temperature for growth (Broeze *et al.*, 1978); specifically, initiation of translation is blocked, but it is possible that this is related to energy levels in the cell. Psychrophiles also show lipid and membrane changes, but in the direction opposite to those for thermophiles, of course. Some enzymes have been purified from psychrophiles, and shown to have activity at lower temperatures, but the studies have not reached the definition of those with thermophiles discussed above.

### ADAPTATION TO PRESSURE

Microorganisms which are exposed to high pressures are found principally in the deep ocean (over 10 000 m; 1160 atm), and in deep oil and sulfur wells. Although there is no question that organisms live at those pressures, it is not certain that this represents a true adaptation, or that these so-called *barophiles* actually require higher pressures. Apparently many common bacteria grow better at somewhat higher pressures,

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depending also on other conditions. An example is *S. faecalis* 9790, which shows maximum growth rate at 100 atm. and 45 °C (Marquis and Matsumura, 1978). It is believed that microbial activity is low in deep waters, which are also cold, although measurements are difficult. High pressure will affect a chemical reaction which results in a change in volume. Among biochemical reactions at pressures found in nature, little change would be expected for most small molecule interactions, DNA is stabilized because its denaturation would result in a small increase in volume, and most proteins appear to be stabilized slightly.

## CONCLUSIONS

From this brief summary, it is apparent that we have only the most elementary understanding of the molecular mechanisms by which microorganisms adapt to their environment. Studies of adaptation to high temperature have been the most popular, and have produced the most definitive results. It is evident now that these remarkable adjustments to physical factors are brought about by subtle changes in molecular architecture.

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